STUDIES ON CARBON DIOXIDE EVOLUTION FROM THE SOIL(Dissertation_全文)

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CHAPTER 1

INTRODUCTION

Soil respiration is a useful index for biological activities in soil through the measurement of O_2-uptake and/or CO_2-evolution, which enables us to evaluate the rate of decomposition of soil organic carbon. Soil respiration is also discussed in relation to the global carbon cycle (Bolin, 1983). Various methods have been devised for the measurement of CO_2-evolution from soil and a number of researchers have conducted the measurement.

Alkaline absorbent methods of measuring CO_2 evolution from soil have been used for a long time. Kirita (1972 a, b, c, d, and Kirita and Hozumi, 1966) made a detailed reexamination of this method, and proposed an improved absorption method using a disc of plastic sponge as absorbent holder. He and some other researchers investigated carbon cycles under various forests using this method.

On the other hand, Jong and Schappert (1972) reported a method of calculation of soil respiration from CO_2 profiles in the soil, based on the theory and application of gas chromatography in a soil aeration research, which had been reported by Tackett (1968).

The infrared gas analyzer (IRGA) has sometimes been used for the measurement of CO_2 evolution from soil after about 1970. Various methods using IRGA have been devised for the measurement of CO_2 evolution (e.g. Edwards and Sollins, 1973, Mathes and Schriefer, 1985, Parkinson, 1981, Sakamoto and Yoshida, 1988), and they were compared with alkaline absorbent methods. But these methods were not so readily applicable to the measurement of soil respiration from multiple plots under various field conditions because of the complexity of their analytical system.

Under laboratory conditions, various complicated methods are available (e.g. Cleve et al., 1979, Nordgren, 1988). The IRGA methods for CO_2 measurement have greatly facilitated the assessment of soil biological activity, and they are superior to the methods using an alkaline absorbent, in terms of both sensitivity and speed.

Application of respiration data taken from experiments under laboratory conditions to soil biomass estimation was first reported by Jenkinson et al. (1976 a b c d, Powlson and Jenkinson, 1976), using the effect of partial sterilization. They also derived biomass estimation from intact soil samples (Jenkinson and Powlson, 1980). Anderson and Domsch (1978) also proposed another method to evaluate soil biomass using the respiration data of glucose-amended soil. Sparling et al. (1981 a, b, c) measured heat

output data in place of respiration. Various applications of these methods were reported by many researchers (e. g. Anderson and Domsch, 1985, Shan-Min et al., 1987, West et al., 1986, Werf and Verstraete, 1987 a, b, c).

The rate of soil respiration is affected by soil temperature, soil moisture, supply of oxygen and organic matter. It is relatively easy to confirm the effect of each of these factors under laboratory conditions (e.g. Orchard and Cook, 1983, Terry et al., 1979). In the field, however, the effects of these factors on soil respiration have not been fully understood and more data under various conditions are required.

As described above, many researchers have measured CO_2 evolution from the soil, and different methods have been devised for its measurement. But improvement and discussion of the methods are still going on because the data of CO_2 evolution are liable to contain various types of "errors", and because simplicity of measurement is very important for its use under various conditions. Also the behavior of CO_2 in the soil has not been well studied in spite of its importance in the soil biotic environment.

Kirita (1971) reported that CO_2 concentration in a chamber, containing CO_2 absorbent, placed on the ground, exerted an influence on CO_2 evolution but this influence was small enough to be applicable to the measurement of the mean rate of soil respiration

under forest. Many of our field measurements were consistent with his, but some were not.

Martens (1987) reported a large difference between two methods of measurement of CO_2 evolution from high pH soils, and attributed the difference to bicarbonate content of the soil and partial pressure of CO_2 of the soil air.

Such a concentration dependence of CO_2 evolution can be caused by both reversible CO_2 sorption in the soil and the suppression of microbial activity under low partial pressure of O_2. But when partial pressure of CO_2 is low (about <2000ppm), because partial pressure of O_2 is not so variable, CO_2 sorption can be the most important cause of concentration dependence.

Sorption, an important property of soil particle surfaces, has been well investigated. But CO_2 sorption in the soil in relation to the measurement of soil respiration has not been well investigated because CO_2 behaves complicatedly in soil.

In this thesis, the factors influencing the variation and error of CO_2 evolution were quantitatively studied, using an improved method for the measurement of CO_2 evolution, and an automatic setup specifically devised for the investigation.

CHAPTER 2

MEASUREMENT OF SOIL RESPIRATION IN THE FIELD: INFLUENCE OF TEMPERATURE MOISTURE, AND APPLICATION OF SEWAGE SLUDGE COMPOST AND AGROCHEMICALS

The soil respiration data discussed in this chapter were obtained under field conditions using a portable IRGA to measure the CO_2 concentration in a small and simple chamber placed on the ground. This method is different from the "air flow method" (in which the use of IRGA is almost standardized (e.g. Edwards and Sollins, 1973, Mathes and Schriefer, 1985)) in that it does not require many valves and long tubes for the measurement of a large number of sample plots. Accordingly, it allows for a relatively easy in situ measurement, although more manual work is required.

We also evaluated the influence of temperature, moisture level, and application of sewage sludge compost and agrochemicals on soil respiration under field conditions.

Materials and Methods

Many of the chambers used for the measurements may be separated into three parts (Fig.2-1 left): the plate, the upper pipe and the lower pipe. The diameter of the pipes was approximately 15 cm and the length was approximately 10 cm for the lower part and

16 cm for the upper part. The other chambers consisted of two parts (Fig.2-1 right): the plate and the pipe, the latter having a diameter of 20 cm and a length of 17-45 cm. The latter chambers were used for the measurement in the experimental field applied with sewage sludge compost, as described below. A small hole was made in the plate, which was stoppered when the internal air was not sampled. An infrared gas analyzer (ZFP5YA31 Fuji Electric), weighing about 6kg with a plastic tube connected to the chamber was used for the measurement of CO_2-concentration.

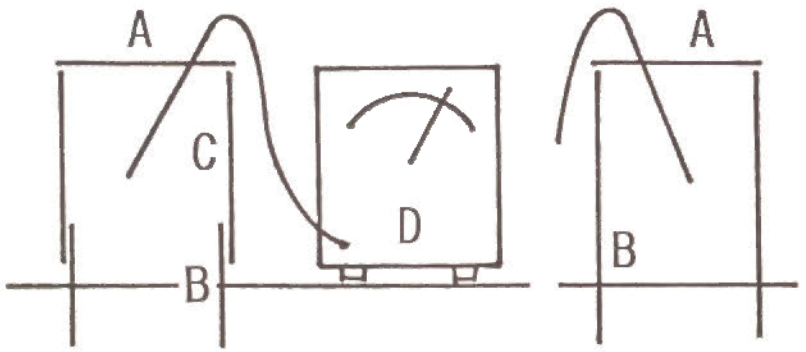

Fig.2-1 Outline of Measurement of CO_2 Evolution from Soil.
A: plastic plate, B C: plastic pipes, D: IRGA (infrared CO_2 gas analyzer)

To measure soil respiration, the lower part of the chamber was forced into the soil at a depth of about 5 cm. The procedure was as follows:

1. Connecting the lower chamber with the upper one, and sealing the top of the chamber with the plate using plastic tape ($\underline{t}$=0).
2. Inserting the connection tube of IRGA into the chamber through the small hole of the plate, and measuring the CO_2-concentration in the chamber.
3. Repeating the above measurement of CO_2 concentration.

The air volume sampled in one measurement was approximately 150 ml, and the concentration measured was corrected by Equation 2-1, where $\underline{c}'$ is the concentration measured, $\underline{c}$ is the corrected value, $\underline{c}_{in}$ is the concentration in the air flowing into the chamber (350ppm or 0ppm with a lime tube for CO_2 absorption), $\underline{r}$ is the ratio of the volume sampled to that of the chamber, and the subscript $\underline{i}$ or $\underline{j}$ indicates the $\underline{i}$th or $\underline{j}$th measurement.

$$\underline{c}_i = \underline{c}'_i + \sum_{\underline{j}=1}^{\underline{i}-1} \underline{r}\,(\underline{c}'_j - \underline{c}_{in}) \qquad \text{Eq.2-1}$$

The extent of the difference caused by air sampling was relatively small in comparison with the variation of the data obtained at different times. The CO_2 concentration $\underline{c}$ (m^3m^{-3}) at time $\underline{t}$ (h) may be expressed by Equation 2-2, where $\underline{p}$ is the basal area of the chamber (m^2), $\underline{q}$ is the volume of the chamber (m^3), $\underline{v}$ is the rate of soil respiration ($m^3m^{-2}h^{-1}$)

and a is the expected value of the CO_2 concentration at t=0.

$$c = vpt/q + a$$
$$= vt/h + a \qquad \text{Eq.2-2}$$

When the chamber is column-shaped, $p/q=1/h$, where h is the height of the chamber.

The field measurements were conducted in the Experimental Farm of Shimane University (Nishi-Kawatsu, Matsue) where the soil has a sandy loam texture, a low organic matter content (almost 2%), a pH (water) value around neutrality (approximately 7) and an undeveloped soil profile due to severe soil disturbance. No crops were planted in the plots for the measurements described in this paper, but the plots were weed-infested.

The following treatments were given: In one set, 500 ml of pesticide consisting of fenitrothion (500ppm), 10 l of fungicide consisting of chlorothalonil (750ppm), 500 ml of herbicide consisting of paraquat dichloride (480ppm), and, in another set, the same chemicals with 5 fold concentration were directly applied to the soil in the experimental plots, each $2m^2$, on Oct.16 in 1985, Apr.26 in 1986, Oct.8 in 1986, Apr.27 in 1987 and Nov.9 in 1987. The measurements of soil respiration were conducted during the period from March, 1987, to January, 1988.

Sewage sludge composts were applied to 4 plots, each about 4 m^2 at rates of 0.5t/10a for 2 plots, and 2.5t/10a for another 2 plots, on April 30th and

October 13th in 1986. Measurements of soil respiration were conducted during the period between April, 1986, and January, 1987.

Soil temperature was measured in each chamber at a depth of 5cm before and after the measurement of the soil respiration. Soil moisture was also measured with samples taken from the plots.

The time course of soil respiration after the application of the above chemicals was also followed in the laboratory in an automatic experimental setup, details of which will be given in a later chapter. The soil used in the laboratory experiment was taken from the same field as that for *in situ* measurements, and the rate of the chemicals applied was the same as that in the field, assuming an affected depth of 10 cm and a bulk density of 1.

Results and Discussion

The chambers used for the measurements were found to be practically impermeable to CO_2. Since the CO_2 concentration in the chamber increased almost linearly with time, and the difference of the temperature before and after a measurement was very small, it was concluded that the closure of the chamber did not affect soil respiration at least for the duration of the period of the measurement.

The results of the measurements indicated that the rate of soil respiration exhibited an arithmetic mean of 15.3, a geometric mean of 11.4, a minimum

value of almost zero, and a maximum value of about 80, (m mol hour^{-1} m^{-2}) (103 plots, 1081 data), the two extreme values being not exceptional in the frequency distribution.

Figure 2-2 shows the annual variation of the means of the rate of soil respiration and soil temperature in the experimental field applied with agrochemicals. Both curves assumed a similar shape with a peak in summer, indicating that soil respiration is affected by temperature.

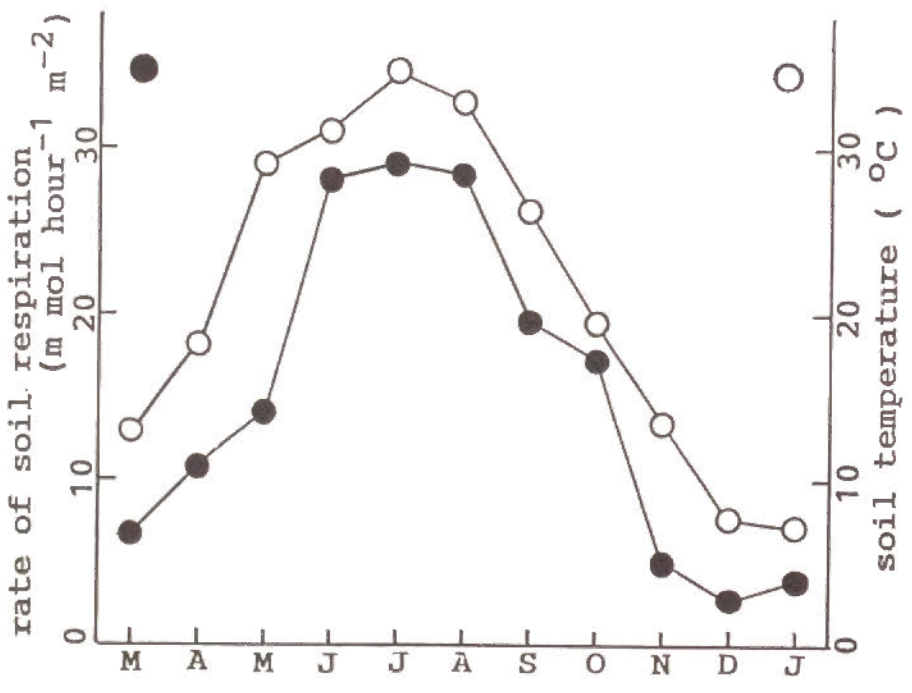

Fig.2-2 Annual Variation of Rate of Soil Respiration and Soil Temperature.

Figure 2-3 shows the relationship between the rate of soil respiration and soil temperature in the field for the test on agricultural chemicals, where the rate scale was made logarithmic. The relationship was linear, with a correlation coefficient of 0.87 (R^2=0.76), indicating that 3/4 of the total variation of soil respiration were caused by soil temperature. The regression line had an intercept of 0.311 and a slope of 0.0337, suggesting that the rate of respiration approximately doubled for each 10^{o}C rise in temperature.

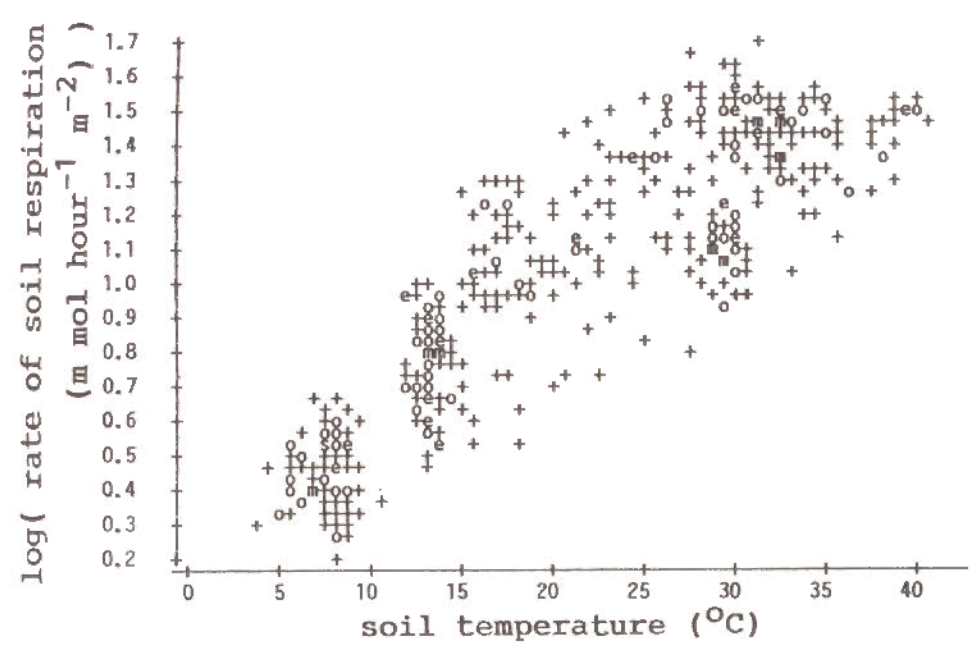

Fig.2-3 Relationship between Rate of Soil Respiration and Soil Temperature. The symbols in this figure indicate the number of data in the same location, as 1: + , 2: o , 3: e , 4: m , 5: s .

Some examples of the mean of soil respiration are about 3 (m mol hour^{-1} m^{-2}) in a desert rangeland (U.S.A.) (Parker et al.,1983), 2 during a secondary succession (Germany) (Mathes and Schriefer, 1985), 16 in a shifting cultivation area (Thailand) (Tulaphitak et al., 1985) and 10 in a warm-temperate evergreen broadleaf forest (Japan) (Kirita, 1971). It was also shown in these reports that soil respiration was markedly affected by temperature. The study we report in this paper shows similar results.

The correlation coefficient between the soil moisture and soil respiration was -0.40, that between the soil moisture and soil temperature was -0.71, and that between the soil moisture and the residual of regression analysis of soil respiration on soil temperature (partial correlation coefficient) was 0.38, which indicates that the soil respiration was stimulated weakly by the soil moisture itself (excluding the influence of the soil temperature). Details of the relationships are shown in Figure 2-4 for the test on agricultural chemicals. Although data fluctuated widely, the figure shows that when soil temperature was maintained within a certain range (15-25 or 25-35^{o}C), soil respiration tended to be enhanced when the soil moisture stayed around 18%. Thus, it appears that soil moisture level certainly affected soil respiration, but its effect was not as conspicuous as that of temperature, presumably because moisture varied in the relatively narrow

range of about 5% to 23%. For that range it did not seem to be conclusively rate-limiting. A strong suppression of soil respiration was observed only at a very low rate (<3%) of soil moisture in one plot in the same farm.

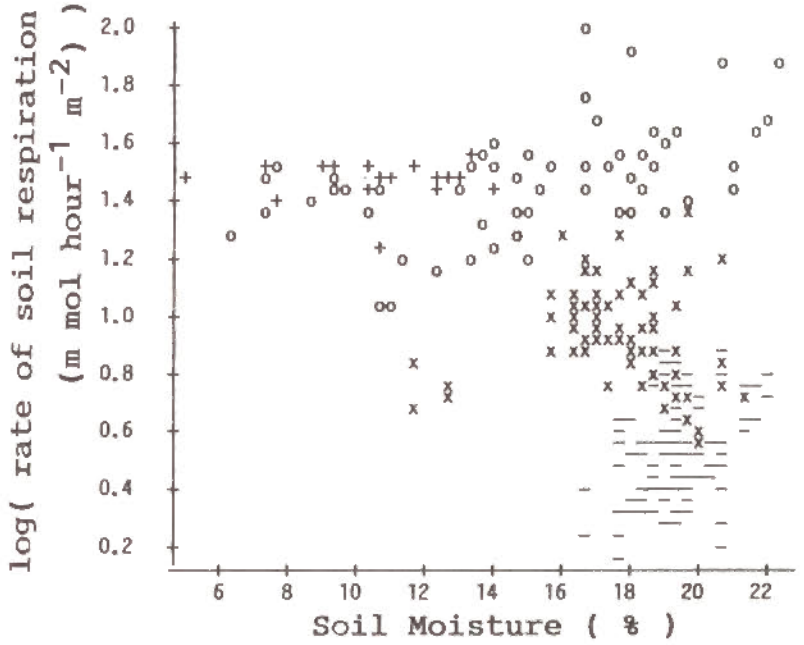

Fig.2-4 Relationship between Soil Respiration, Soil Moisture and Soil Temperature.
legend: - < 15^{o}C
x 15^{o}C =< , < 25^{o}C
o 25^{o}C =< , < 35^{o}C
+ 35^{o}C =<

Table 2-1 gives the results of the analysis of variance and the comparison of the means of the rate of soil respiration in the experimental field treated with sewage sludge compost, where a significant difference was observed between the mean rate of respiration in the plot amended with 2.5t/10a of the compost and those in the others. There was, however, no significant difference between the plot amended with 0.5t/10a and the control plot.

Table 2-1 Results of Analysis of Variance and Comparison of the Means of the Rate of Soil Respiration in the Experimental Field Treated with Sewage-Sludge-Compost.

		log[@]
Number of data	214	214
R^2	0.091	0.089
F-value	10.6	10.2
Significant Prob.	0.001	0.001

plots	means	log[@] means
2.5t[#]	13.8 a*	11.7 a*&
0.5t	9.6 b	7.9 b
0.0t	9.3 b	7.5 b

@ Analysis after logarithmic transformation. # weight of application per 10a. * m mol hour^{-1} m^{-2}, The means in each column followed by the same letter did not differ significantly (P>0.05). & geometrical means.

The rate of soil respiration is considered to be proportional to the amount of organic matter applied. However in the field, as shown in Table 2-1, it was very difficult to validate this assumption because the variability of the data as affected by the temperature, moisture level and plant activity tended to be greater than the accuracy required for the confirmation.

The relationship between weed growth (top weight) and soil respiration was also evaluated in some fields, and a low but positive correlation was observed. Weeding of the experimental plot often, but not always, decreased soil respiration rate. Although plants contributed to most of the organic carbon in the field, the influence of plant growth, or of weeding, on soil respiration was relatively limited because the top weight may not be an adequate index of root activity. In the plots used in this study, the top weight did not give indication of the distribution of the root. Root weight, which may be an adequate index for organic carbon supply, cannot be easily measured, especially in the field.

Table 2-2 shows the results of the analysis of variance and the comparison of the means of the rate of soil respiration in the experimental plots to which pesticide, fungicide and herbicide had been applied. The probability of significance in the table indicates that there was no difference between the plots treated or not treated with agricultural chemi-

cals.

Table 2-2 Results of Analysis of Variance and Comparison of the Means of the Rate of Soil Respiration in the Experimental Field applied with Pesticide, Fungicide or Herbicide.

		log@
Number of data	385	385
R^2	0.009	0.011
F-value	0.55	0.71
Significant Prob.	0.770	0.641

plots	means	log@ means
fenitrothion pesticide 1#	16.2*	12.1*&
fenitrothion pesticide 5	15.9	11.1
chlorothalonil fungicide 1	13.0	9.0
chlorothalonil fungicide 5	15.8	11.8
paraquat dichloride herbicide 1	15.1	11.5
paraquat dichloride herbicide 5	15.4	11.2
non	15.8	11.6

@ Analysis after logarithmic transformation. # 1;application at standard concentration, 5:five fold concentration. These agricultural chemicals were applied several times for about 3 years. * m mol $hour^{-1}$ m^{-2}. & geometrical means.

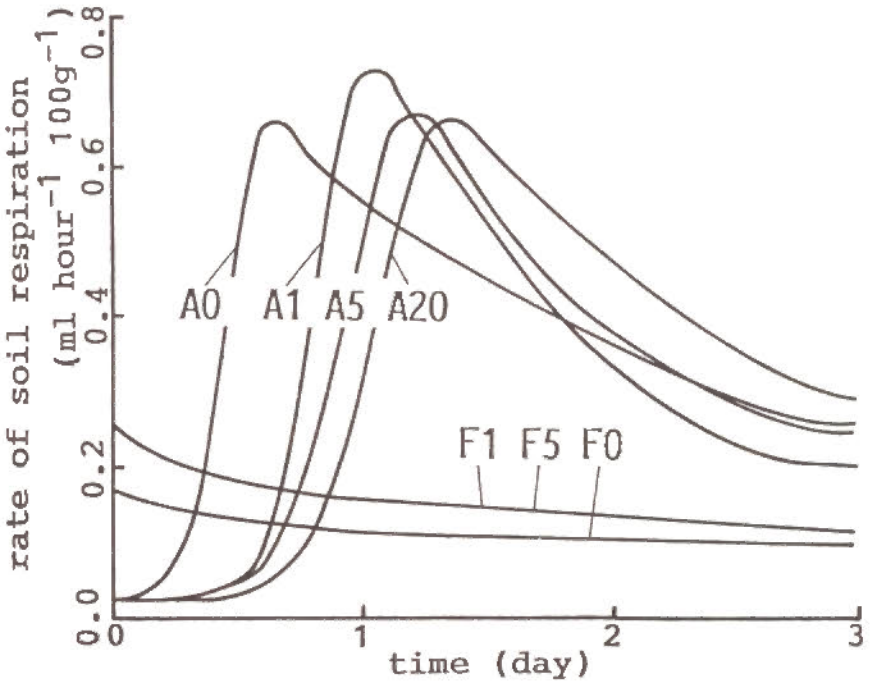

Fig.2-5 Influence of Fungicide Application on Soil Respiration under the Laboratory Condition. A: inoculation of 0.1g of fresh soil into 100g of autoclaved soil, A0: application of 5ml of water, A1: application of 5ml of chlorothalonil fungicide at 750ppm, A5: 5 fold concentration, A20: 20 fold concentration, F: 100g of fresh soil, F1: application of 5ml of the same fungicide to 100g of fresh soil. F5: 5 fold concentration. The rate of soil respiration for a period of 4-7 days is only gradually reduced.

Figure 2-5 shows the influence of fungicide on soil respiration under laboratory conditions. The influence on unsterilized soil was negligible as

reflected in the shape of the curve of time course, and the curve of fresh soil was lower than the soil treated with fungicide. However, the data were often too variable to allow a comparison among different samples without a statistical analysis. The influence on sterilized soil was observed as shown in the figure, where the higher the concentration of the fungicide, the longer the time lag before a steep rise. Similar results were also obtained with other soil samples, but the effects of herbicide and pesticide were not observed. All the laboratory experiments were conducted at 25^{o}C and the soil moisture level was kept at about 20%.

Because soil respiration is an index of the total biological activity in the soil, it can remain relatively constant even when some organisms are inactive. (if the others remain active at the same time.) Therefore the soil respiration is markedly affected by a factor relevant to all the organisms, such as temperature, but only weakly by a factor that is relevant to only particular organisms, such as pesticides.

A steep rise of soil microbial activity after biocidal treatment of soil was closely studied by some workers, e.g. Jenkinson et al. (1976 a b c d, Powlson and Jenkinson, 1976). But in our study, a steep rise or even any change of soil respiration was not observed in the field applied with a standard or

a 5-fold dose of agrochemicals. Sato (1981) mentioned that agrochemicals affected soil respiration minimally because soil respiration was concerned with all microbes, while nitrification was conducted only by a specific group of microbes that were affected strongly by agrochemicals.

Summary

Soil respiration data were obtained using a portable Infrared Gas Analyzer to measure increments of the CO_2 concentration in a small chamber placed on the ground. The chamber is made of plastic plate and pipe(s), several liters in volume, columnar in shape, and open at its bottom to be forced into the soil by about 5cm. The IRGA weighs about 6kg and has a plastic tube connecting it to the chamber and a suction pump for air sampling.

The rate of respiration showed a geometrical mean of 11.4 (m mol hour^{-1} m^{-2}) and ranged from almost zero to about 80 (m mol hour^{-1} m^{-2}) in the experimental field in the farm of Shimane University (103 plots, 1081 data). The rate was stimulated strongly by the temperature (R^2=0.76, Q_{10}=2.2, 4^{o}C<, <40^{o}C), and weakly by the moisture level (partial correlation coefficient: 0.4) and input of organic matter (sewage sludge compost). But the influence of agrochemicals (standard and 5 fold dose of fenitrothion pesticide, chlorothalonil fungicide and paraquat dichloride herbicide) was too small to be de-

tected under both the field and laboratory conditions.

CHAPTER 3

AUTOMATIC MEASUREMENT OF CO_2 EVOLUTION FROM MULTIPLE SAMPLES IN SMALL CHAMBERS.

As CO_2 is a universal product of living organisms, the measurement of CO_2 evolution is important for analyzing biological activity. Recently, the use of an infrared gas analyzer (IRGA) for CO_2 measurement has facilitated the assessment of soil biological activity, especially under laboratory conditions. The IRGA method for CO_2 measurement is more convenient than the methods using an alkaline absorbent, in terms of sensitivity and speed. In this chapter, the automatic setup using IRGA and the computer and program used for the continuous measurement of CO_2 evolution from multiple samples are described.

Materials and Methods

Figure 3-1 schematically illustrates the automatic setup for the measurement of CO_2 evolution. A soil sample is weighed into a chamber with an air outlet tube connected to the IRGA (ZFP5YA31, Fuji Electric). The values of CO_2 concentration measured with IRGA are conveyed to a personal computer (PC-9801, NEC), through an analog-digital converter (AB98-05, Adtec System Science). The chamber where the CO_2 concentration is measured is selected by solenoid valves, which, together with the suction

pump of the analyzer, are controlled by the computer through relays.

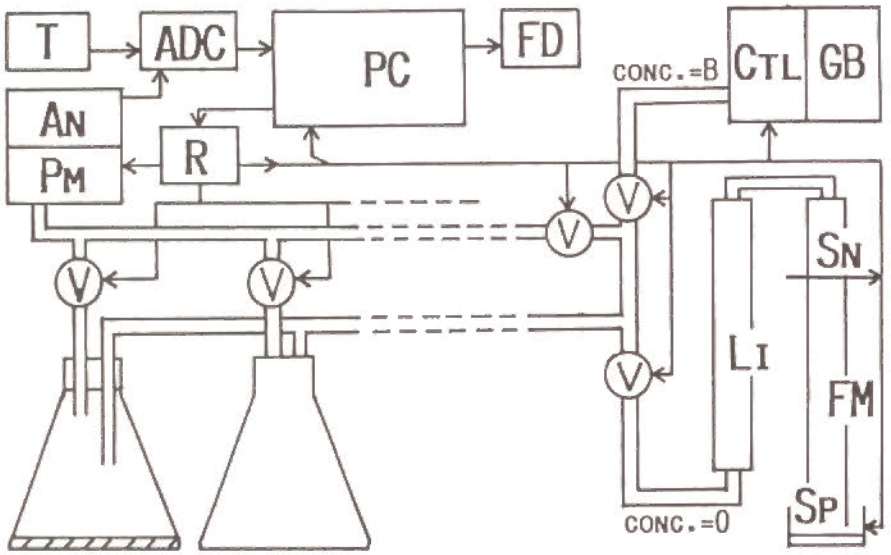

Fig.3-1. Outline of the automatic setup for the measurement of CO_2 evolution. FM: flow meter with Sp and Sn, Sp: apparatus by which a soapsuds-film adheres to the entrance end of the glass tube, Sn: sensor for optical reflection, Li: lime tube for CO_2 absorption, GB: gas bomb with known concentration B of CO_2, Ctl: pressure controller, V: solenoid valve, Pm: pump for suction, R: relay, An: infrared CO_2 gas analyzer, T: thermometer, ADC: analog-digital-converter, PC: personal computer, FD: floppy disk.

Before and after the air of a certain chamber is sucked, CO_2-free air is introduced to clean up the tube and the analyzer cell in order to enhance the sensitivity and accuracy of measurement. An air flow meter is installed at the entrance end of a lime column. The lime column is used for absorption of CO_2 in the air. The air-flow meter consists of a glass tube, an apparatus by which a soapsuds-film adhered to the entrance end of the glass tube, and a sensor for optical reflection of the film. The reading of the meter is monitored by the computer.

For the measurement of negative CO_2 evolution (CO_2 sorption), gas with a known concentration of CO_2 is supplied from a gas bomb.

The data measured are stored in a floppy disk to be retrieved for various calculations and graphics.

Carbon dioxide evolution was calculated by Equation 3-1, where C' is the CO_2 concentration in a chamber, $\Delta C'$ is the difference of C' between consecutive measurements, M and $\overline{C}$ are, respectively, the volume of the air sucked and the mean CO_2 concentration in the air in the last suction, and V is the volume of the chamber. Based on the equation, CO_2 evolution of a sample (X) is represented as the sum of the increment of CO_2 in the chamber ($\Delta C'$ V) and the CO_2 output at the last measurement ($\overline{C}$ M). If the CO_2 concentration of the input air is not zero but B (case(conc.=B) in Figure 3-1), B M must be subtracted from Equation 3-1 (Equation 3-2). Al-

though Equation 3-1 requires at least two measurements of concentration C', the time interval between the two measurements can be set arbitrarily, provided that the concentration remains in the measurable range of the IRGA.

$$X = \overline{C}\ M + \Delta C'\ V \qquad \text{Eq.3-1}$$

$$X = \overline{C}\ M + \Delta C'\ V - B\ M \qquad \text{Eq.3-2}$$

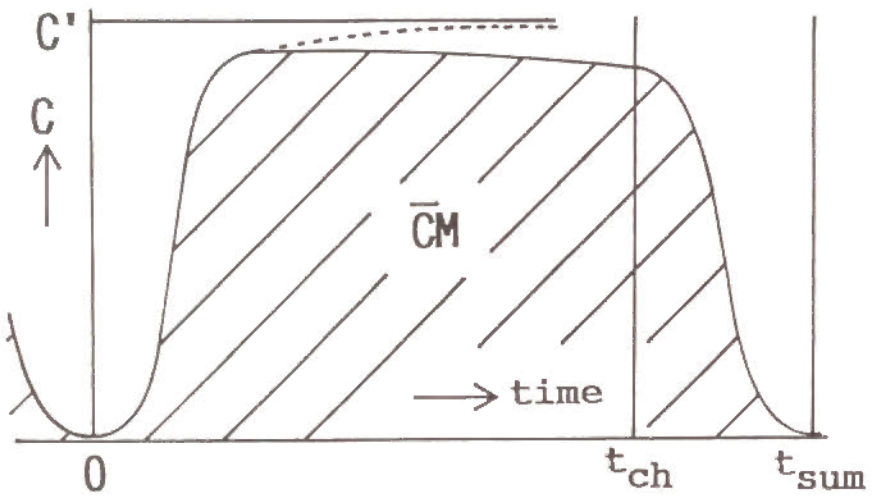

Fig.3-2. Evaluation of CO_2 concentration C' and total output of CO_2 $\overline{C}$ M from the sequential data of CO_2 concentration measured for a chamber. C: CO_2 concentration, t: time, t_{ch}: duration of suction from a chamber, $t_{sum} - t_{ch}$: duration of direct suction of CO_2-free gas.

The method of evaluation of C' and $\overline{C}M$ based on

the sequential data of the concentration measured is illustrated in Figure 3-2, where the broken line indicates the ideal case when C' is constant during the measurement, and the solid line indicates the data in the actual measurement, in which C' decreases by the introduction of CO_2-free air. These data were obtained using the input air with a known CO_2 concentration (e.g. 1960 ppm). The concentration C' in the chambers with solenoid valves (AB21022, CKD) in the apparatus shown in Figure 3-1 was found to be about 1.04 times that of the output air 11 to 12 seconds after the start of suction of the air in a chamber, although this value varied depending on the design of the apparatus (e.g. type of valve used). Accordingly these parameters must be determined for each setup before use. The total output of CO_2 ($\overline{C}$ M) is as follows:

$$\overline{C}\ M = \frac{M}{t_{ch}} \int_0^{t_{sum}} C\ dt \qquad \text{Eq.3-3}$$

where t_{ch} is the duration of suction from a chamber, and t_{sum} is the sum of t_{ch} and the duration of direct suction of CO_2-free gas.

The parameters used in our experiments were as follows: the range of C was 0-2000 (ppm), V≒1.1 (l), t_{sum}=40 (sec.), t_{ch}=30 (sec.), M≒260 (ml), and the interval between two consecutive measurements of C' was set from several minutes to several hours and controlled by the computer.

Results

Carbon dioxide evolution in an empty chamber was confirmed to be almost zero. The results of measurement of CO_2 absorption with a KOH solution placed in this apparatus were similar to those obtained by a titration method.

The results of the measurement of soil respiration using this apparatus were reported previously (Chapter 2) and some other data, e.g. the effect of partial sterilization and nutrient amendment on soil respiration, and CO_2 sorption by soil, will appear in the chapters to follow.

Summary

An automatic setup using an infrared gas analyzer, a computer and a program for continuous measurement of CO_2 evolution from multiple samples was devised.

A soil sample is weighed into a chamber with an air outlet tube connected to IRGA. The values of CO_2 concentration measured with IRGA are conveyed to a personal computer, through an analog-digital converter. The chamber where the CO_2 concentration is measured is selected by solenoid valves, which, together with the suction pump of the analyzer, are controlled by the computer through relays.

CO_2 evolution was calculated by the following equation, where C' is the CO_2 concentration in a chamber, $\Delta C'$ is the difference of C' between consecutive measurements, M and $\bar{C}$ are, respectively, the volume of the air sucked and the mean CO_2 concentration in the air in the last suction, and V is the volume of the chamber.

$$X = \bar{C}\, M + \Delta C'\, V$$

CHAPTER 4

CHANGES OF SOIL RESPIRATION AFTER PARTIAL STERILIZATION WITH AUTOCLAVING OR ADDITION OF AGROCHEMICALS

Partial sterilization, which is the result of biocidal treatment, is known to cause a steep rise in microbial activity in the soil. Specifically, an increase in available nitrogen after drying, burning and freezing of soil has found practical application in agriculture and has been well investigated. On the other hand, the relationship between soil respiration and partial sterilization has not been so well understood. Although the effect of soil fumigation was closely studied by Jenkinson and Powlson (1976) and some other researchers, generally speaking, the influence of agrochemicals on soil respiration has not been well investigated. In one of our previous studies, a steep rise or even any change of soil respiration was not observed in the field applied with a standard or a 5-fold dose of fungicide, pesticide and herbicide (cf. Chapter 2).

Consequently, the extent and property of biocidal treatment are thought to be important for the understanding of the effect of partial sterilization. In this paper we studied quantitatively the influence of sterilization of soil samples on soil respiration

under laboratory conditions.

Materials and Methods

The soils examined were the following:

Sample 1 was a sandy loam soil from upland in the Experimental Farm of Shimane University. It had a low organic carbon content (1.5%) and a pH (H_2O) value of 6.3, and was sampled from the control plot in the previous experiment (cf. Chapter 2).

Sample 2 was a light clay soil from a paddy field in the Experimental Farm of Shimane University. It had a low organic carbon content (1.3%) and a pH (H_2O) value of 5.9.

Sample 3 was an andosol from a forest on the foot slope of a volcano Mt. Daisen, Tottori Pref., which had a high organic carbon content (7.2%) and a weakly acidic pH (H_2O) (5.8).

Soil moisture contents used for the experiments were set approximately at field capacity, i.e. 20% for the light clay, 15% for the sandy loam, and 38% for the andosol (on wet soil basis).

The soils used in the experiment to compare the mean rate of soil respiration were homogenized by passing them through a 2mm sieve.

The setup used in the experiment was described in a previous chapter (Chapter 3). Unless otherwise stated, all the measurements were conducted at 25°C in an air-conditioned room.

Chloropicrin and metam ammonium were added to

100g of soil in glass chambers, using a micro-syringe. The soil was fumigated for 1 to 3 days after closing the chamber. And then these fumigants were removed by evacuation.

In this paper partial sterilization was simulated by mixing autoclaved (120^{o}C, 30min.) soil with fresh soil.

Results and Discussion

Figure 4-1 shows the change of soil respiration after mixing autoclaved and fresh soil. The soil used for this figure is the light clay soil. Similar results were obtained with the other soil samples. When the weight ratio of autoclaved soil to total soil was more than 50%, a steep rise in the rate of soil respiration was observed after a certain time lag. When the ratio was 10% to 30%, the rate of soil respiration was relatively constant and showed a pattern similar to that of the fresh soil itself, while the mean rate of soil respiration of the mixed soil was larger than that of the fresh soil, as shown in Table 4-1.

The rates of soil respiration after application of a standard, 5-fold or 20-fold dose of chlorothalonil, a fungicide, were also relatively constant, and a little higher than that of the control soil under laboratory conditions. The data were, however, often too variable to allow a comparison among the mean rates of these treatments without a statistical

analysis of many data (cf. Chapter 2). The influence of a weak biocide treatment, as in a contamination with ordinary fungicide, on soil respiration was similar to that of <10% sterilized soil and too small to be detected unless carefully statistically treated.

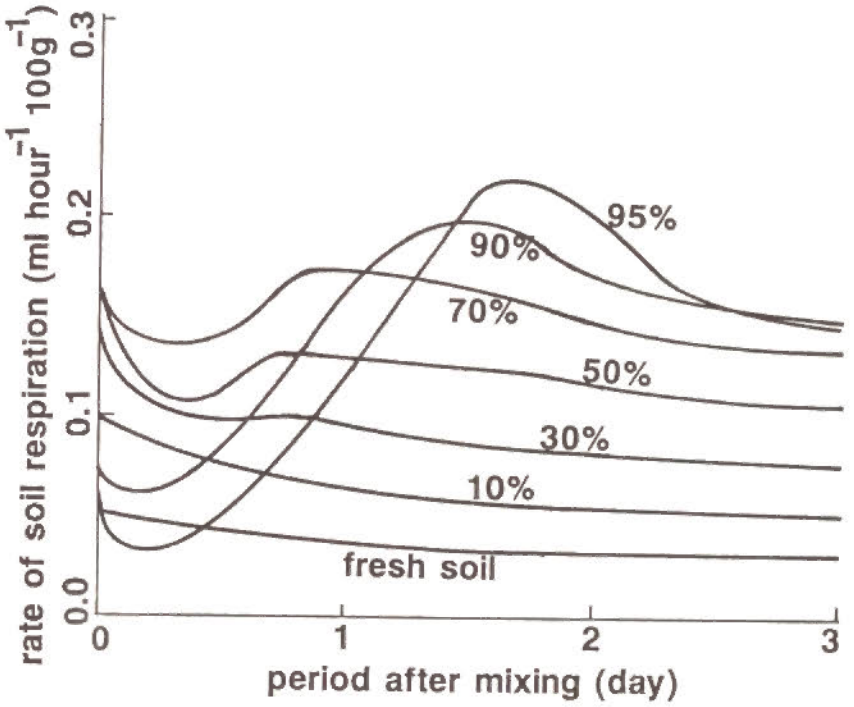

Fig.4-1 Change of soil respiration (CO_2 evolution) after mixing autoclaved and fresh soil, for simulation of partial sterilization. * the weight ratio of autoclaved soil to total soil. The soil used for this figure is the light clay soil.

Table 4-1. Mean rate of soil respiration after mixing autoclaved and fresh soil for the simulation of partial sterilization.

	weight ratio of autoclaved soil to total soil		
	0%	10%	30%
sandy loam	2.08*	2.40	3.42
light clay	0.86	1.41	2.24
andosol	2.36	3.18	5.35

* CO_2 evolution during first 4 day (ml/day)

The influence of soil fumigation on soil respiration is shown in Figure 4-2 and Table 4-2. The curves in Figure 4-2 have a conspicuous peak and they are similar to the curve for the soil which was sterilized 95% in the simulation study shown in Figure 4-1. The soil used for this figure is the light clay soil. Similar results were observed with the other two soils. In contrast, the application of metam ammonium, a mild fumigant, gave curves similar to those with 10% to 30% sterilized soil (see Table 4-1).

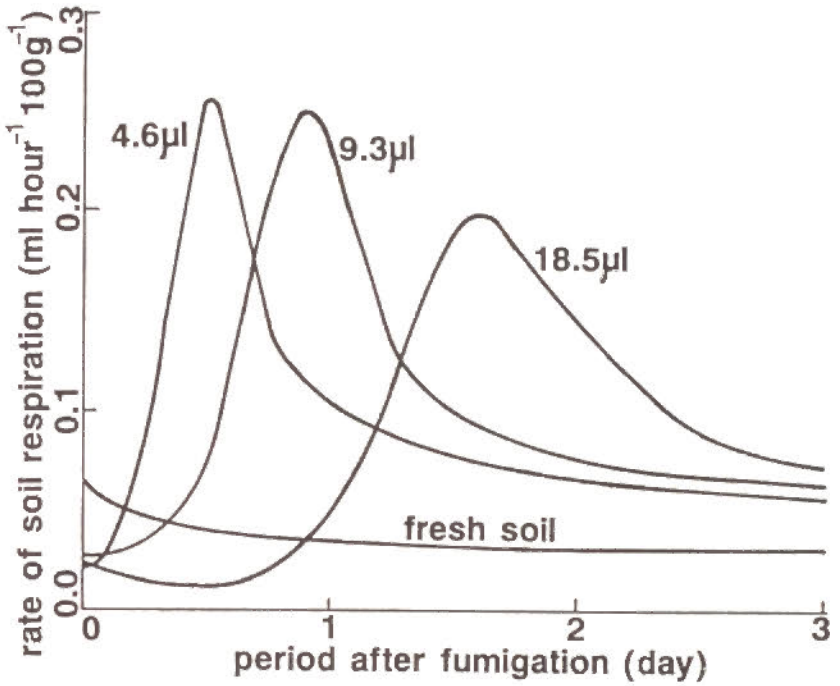

Fig.4-2 Change of soil respiration (CO_2 evolution) after soil fumigation with chloropicrin. * dose (µl / 100g-soil). The soil used for this figure is the light clay soil. Fumigation period was 3 days.

The results shown in Table 4-2 were supported by our plate count data of bacteria using the light clay soil under laboratory conditions, i.e. the ratio of the colony-forming units in soil after fumigation to those in untreated soil (about 10^8 cfu/g-soil) was

only about 7 % with chloropicrin, but about 50% with metam ammonium (Yamamoto et al., currently unpublished).

Table 4-2 gives the mean rate of soil respiration for the metam ammonium treatment. In this table a clear difference is seen between untreated and treated only for the sandy loam soil. There is no difference for the andosol. This condition may be explained by the fact that soil microbes in the sandy loam soil may be inhabiting in large soil pores into which the fumigant is more readily introduced.

In this manner, the conditions for an effective fumigation may be searched for by a measurement of soil respiration.

Table 4-2. Influence of soil fumigation with metam ammonium on soil respiration

fumigation period	1 day		3 days		
dose (μl/100g-soil)	5	10	5	10	untreated
sandy loam	2.08*	2.29	2.12	2.30	1.63
light clay	1.06	1.16	1.15	1.23	1.03
andosol	2.03	2.05	2.01	**	2.00

* CO_2 evolution during first 4 day (ml/day); ** not measured

When a steep rise of soil respiration was observed, the length of time before the rise was dependent on the intensity of the sterilization treatment. It was shown that when more fumigant was applied (Table 4-3), or when more autoclaved soil was mixed (Table 4-4), the longer was the length of time before a steep rise of soil respiration. Table 4-5 gives a similar result for varied sizes of fresh soil or inoculum mixed with sterilized soil, where the smaller the inoculum size, the longer the time lag before a steep rise of soil respiration. This result may not be due to the special nature of soil microbes, for Shida et al. (1975) reported a reduction of the lag time with a larger inoculum size with pure cultures of Escherichia coli, Bacillus subtilis and others.

Table 4-3. The time lag up to the peak of soil respiration after the application of fumigant chloropicrin

fumigation period	1 day			3 days		
dose (μl/100g-soil)	4.6	9.3	18.5	4.6	9.3	18.5
sandy loam	0.3*	0.3	0.5	0.3	0.5	0.7
light clay	0.5	0.7	1.2	0.5	0.9	1.6
andosol	1.2	1.7	2.9	1.5	2.1	>3

* days

Table 4-4. The time lag up to the peak of soil respiration after mixing autoclaved and fresh soil

	weight ratio of autoclaved soil to total soil			
	50%	70%	90%	95%
sandy loam	0.4*	0.6	0.7	0.7
light clay	0.7	0.9	1.4	1.7
andosol	1.2	1.2	1.5	2.0

* days

The incubation temperature clearly affected the time lag, for the time lag at 15°C was 1.8 times longer than that at 25°C, as given in Table 4-5. The cause of difference among soils is not well known, but the distance between proliferating microbes and nutrient (dead microbes), which is in turn affected by soil texture and soil micro structure, may be a primary factor of the difference.

Table 4-5. The time lag up to the peak of soil respiration after mixing autoclaved and fresh soil (inoculation of fresh soil to autoclaved soil)

	weight ratio of fresh soil to autoclaved soil		
	0.5%	0.1%	0.02%
at 25°C	2.9*	3.6	4.6
at 15°C	5.2	6.3	8.1

* days

Summary

Changes of soil respiration after partial sterilization with autoclaving or addition of chemicals were measured under laboratory conditions at 25°C.

Partial sterilization was simulated by mixing autoclaved and fresh soil. When the weight ratio of autoclaved soil to total soil was about 10 to 30%, the rate of soil respiration was relatively constant and the curves were similar to that of the fresh soil itself.

When fumigant, chloropicrin was applied, or when the weight ratio in the above simulation was above 50%, soil respiration was suppressed temporarily, and then stimulated after 0.3-3 days. The higher the concentration of the chemicals, or the more the ratio of autoclaved soil, the longer the time lag before a steep rise of soil respiration.

When a small amount of fresh soil was inoculated to the autoclaved soil, the size of the inoculum decreased the time lag before the steep rise of soil respiration.

CHAPTER 5

CONCENTRATION DEPENDENCE OF CO_2 EVOLUTION FROM SOIL IN A CHAMBER WITH LOW CO_2 CONCENTRATION (<2000ppm): CO_2-DIFFUSION/SORPTION MODEL IN SOIL

Under field conditions, when CO_2 concentration is measured with an infrared gas analyzer (IRGA) in a small chamber placed on the ground, the relationship among CO_2 concentration $\underline{c}$ (m^3m^{-3}), time $\underline{t}$ (hours), basal area of the chamber $\underline{p}$ (m^2), volume of the chamber $\underline{q}$ (m^3) and the constant rate of soil respiration $\underline{v}$ ($m^3m^{-2}hours^{-1}$), may be expressed by Eq.5-1 or Eq.5-2 (Figure 5-1, Curve A).

$$d\underline{c}/d\underline{t}=\underline{vp}/\underline{q} \qquad \text{Eq.5-1}$$

or $$d\underline{c}/d\underline{t}=\underline{v}/\underline{h} \qquad \text{Eq.5-2}$$

As the chamber is column-shaped, $\underline{p}/\underline{q}=1/\underline{h}$, where $\underline{h}$ is the height of the chamber (Chapter 2). But in some fields, $d\underline{c}/d\underline{t}$ is not constant but decreases during the measuring period (1 to 2 hours), even at a low CO_2 concentration in the chamber (<2000ppm) (Figure 5-1, curve B).

Carbon dioxide evolution was also measured under laboratory conditions, using the method reported previously (Chapter 3). In this experiment, soil respiration was found to depend on CO_2 concentration in the chamber, in such a way that CO_2 evolution was stimulated by a decrease of CO_2 concentration.

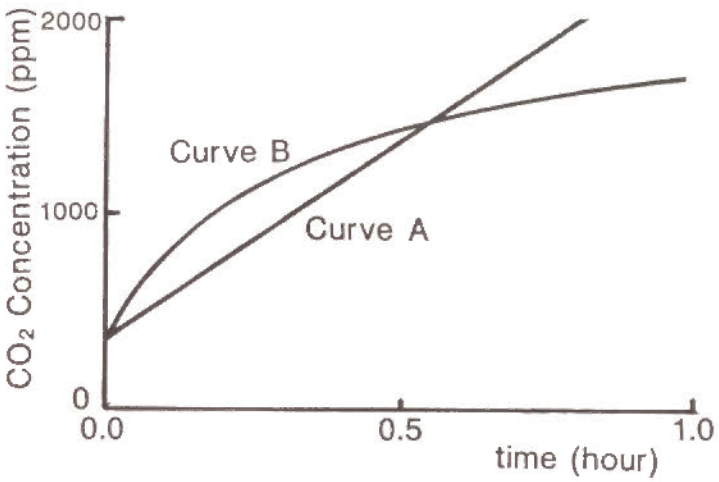

Fig.5-1. Relationship between CO_2 concentration C and time t in a small chamber placed on the ground.

In this chapter, concentration dependence of CO_2 evolution was studied to remove a source of "error" in the measurement of CO_2 evolution and to study the behavior of CO_2 in the soil. CO_2 diffusion and sorption in the soil was discussed briefly in this chapter as a cause of a concentration dependence of CO_2 evolution.

Materials and Methods

The method for measuring CO_2 evolution using IRGA under the field condition was reported in Chapter 2.

The phenomenon of concentration-dependence of CO_2 evolution from a field soil may be expressed by Eq.5-3 (Figure 5-1, Curve B).

$$dc/dt = v/h - k(c-a) \qquad \text{Eq.5-3}$$

where a is the CO_2-concentration at $t=0$ and k is an appropriate constant related to the diffusion coefficient. This model means that CO_2 evolution, (which is constant without the chamber for the measurement), is suppressed by an increase of CO_2 concentration in the chamber, $c-a$.

In order to fit the measured data to the above model (Eq.5-3), first, the differential equation was solved using a formula (Ono and Kitajima, 1981), as shown in Eq.5-4, and then a non-linear least square method was applied using a library program (SSLII, Fujitsu).

$$c=(v/kh)(1-\exp(-kt))+a \qquad \text{Eq.5-4}$$

Errors included in the estimated values of v and k were evaluated by a computer simulation technique and the Monte Carlo method. We also assumed the normal distribution of the error of CO_2 concentration measurement, the technique being similar to the one reported previously (Naganawa and Hattori 1984).

Figure 5-2 shows a schematic pathway of CO_2

diffusion and sorption, where M is the rate of respiration of soil organisms, Ps and Pa are the partial pressures (concentrations) of CO_2 in soil and atmosphere, respectively. Ss is the volume of CO_2 sorption (stock) in the soil which is a function of Ps, D is a sort of CO_2 diffusion coefficient through soil, and x is the rate of CO_2 evolution from soil.

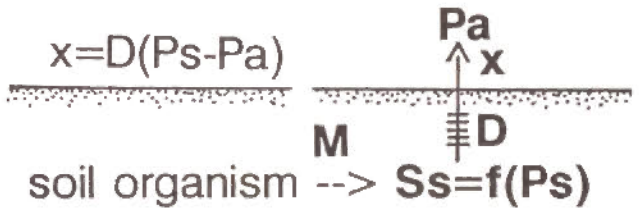

Fig.5-2. Schematic pathway of CO_2 diffusion and sorption from soil organisms to atmosphere. M: rate of respiration of soil organisms, Ps or Pa: the partial pressures (concentrations) of CO_2 in soil or atmosphere, respectively, Ss: volume of CO_2 sorption (stock) in the soil which is a function of Ps, D: a sort of diffusion coefficient of soil, and x: rate of CO_2 evolution from soil.

As regards the values of Ss and D in Figure 5-2, the following 4 cases may be assumed:

Case 1. D≒0 (low diffusion rate).

Case 2. Ss≒0 (small sorption volume).

Case 3. D is very high and Ss is considerably high.

Case 4. D is medium and Ss is considerably high.

In Case 1, Because Ps>>Pa for measurable x, Ps-Pa is almost constant for variable Pa. Consequently, x is also almost constant. (no concentration dependence). In Case 2, x is almost equal to M (no concentration dependence). In Case 3, Ss≒Function(Pa) because Ps≒Pa, and $x=Ss_1-Ss_0+M$, where subscripts 1 and 0 mean the present and the last status, respectively. Accordingly, $x = Function(Pa_1) - Function(Pa_0)+M$. When the function can be approximated to be linear with respect to Pa, $x = Function(Pa_1 - Pa_0)$ (dependence on the difference between Pa). In Case 4, Ps can be considered to be a constant, because it is buffered by Ss. Accordingly x=D(Ps-Pa) can be transformed to x=b-D(Pa-a), assuming appropriate constants a and b, which has the same form as Eq.5-3 (concentration dependence).

Because this explanation is not quantified and ignores the heterogeneity of the soil, its application to the practical data is difficult. But to show the validity of the reasoning qualitatively, CO_2 evolution from a light clay soil (C 1.3%; pH(H_2O) 5.9) to the air in the chamber, as shown in Figure 5-3, was measured using an automatic CO_2 measuring

setup reported in Chapter 3. In this measurement, to observe the concentration dependence of CO_2 evolution, CO_2 concentration in the chambers was regulated by changing the volume of CO_2 free gas flown into the chambers. The CO_2 evolution and the CO_2 concentration were also measured using either a loosely or compactly packed andosol derived from volcanic ashes of Mt. Daisen, a volcano in Tottori Pref., which had a high organic carbon content (7.2%) and a weakly acidic pH (H_2O) (5.8).

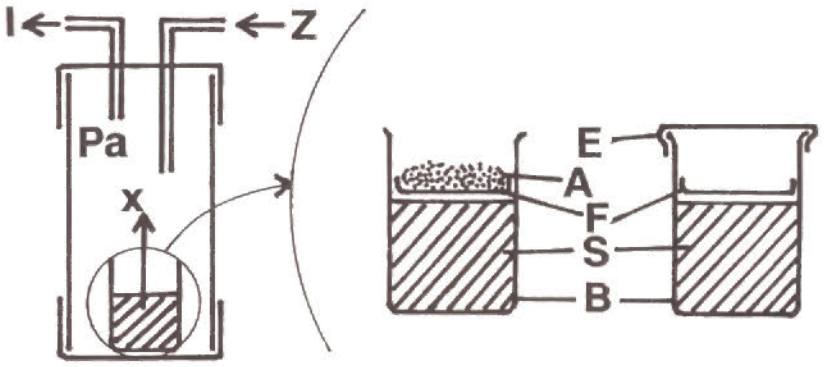

Fig.5-3. Outline of simulation experiment of sorption /diffusion model. I: air exit into infrared CO_2 gas analyzer with suction pump, Z: entrance of CO_2 free air, Pa partial pressure (concentration) of CO_2 in the air in the chamber which was regulated by changing the volume of CO_2 free air flown into the chamber, x: rate of CO_2 evolution from soil to the air in the chamber, B: 50ml glass beaker, S: 50g of soil sample amended with 0.3g of glucose for stimulating CO_2 evolution, F: filter paper, A:activated alumina as an adsorbent of CO_2, E: polyethylene film (0.03mm) for controlling CO_2 diffusion. right: simulation experiment of Case 4, middle: that of Case 3.

Table 5-1. Fitness test of the concentration dependence model under the field condition, i.e. test of the residuals of (measured value - estimated value) using the non-linear model (Eq.3 and 4) and the linear model (Eq.1 and 2).

t / t_{max}		0-1/6	1/6-3/6	3/6-5/6	5/6-1
Exp.P.S[&] ('87)	non-linear	2 (6)*	-4 (17)	4 (17)	-1 (6)
	linear	-7 (31)	5 (39)	11 (25)	-9 (26)
Number of data		433	433	433	433
Kyo.Shi.[#] ('83,'84)	non-linear	-1 (5)	3 (14)	-6 (24)	3 (12)
	linear	-39 (48)	53 (69)	38 (52)	-50 (68)
Number of data		454	454	415	454

*: mean (standard deviation) of the residuals, (ppm). &: the experimental plots applied with agro-chemicals reported previously (Naganawa et al., 1989a) located in Experimental Farm of Shimane University. #: measured in Kyoto Pref. and Shizuoka Pref.

Results and Discussion

Table 5-1 shows the fitness test of the model of concentration dependence (non-linear model, Eq.5-3 and 4) for the field data, i.e. residual test (the value measured minus the value estimated by the above model). In this table, most of the data of measured CO_2 evolution fits this non-linear equation. But, as a result of a computer simulation, the estimated value of $\underline{v}$ has a larger error than that estimated by a linear regression analysis. This indicates that the applicability of this equation is limited to the data for which the linear equation may not be appropriated. Linear regression analysis is a better method for the data from the experimental plots reported in Chapter 2, and which are shown in the upper part of Table 5-1. On the other hand, non-linear equation appears to be better applicable for a comparison of the data described in the lower part of the same table.

Figure 5-4 shows the changes of the rate of CO_2 evolution and CO_2 concentration. Figure 5-4E (Case 4) shows the result of the simulation experiment as shown in Figure 5-3 (right), where a medium rate of diffusion was simulated by covering the soil-containing beaker with a thin plastic film permeable to CO_2. A considerable value of CO_2 sorption by making the contents of the air phase high under the plastic film in the beaker. Similar curves are shown in Figure 5-4T (Case 4), where the soil used was the

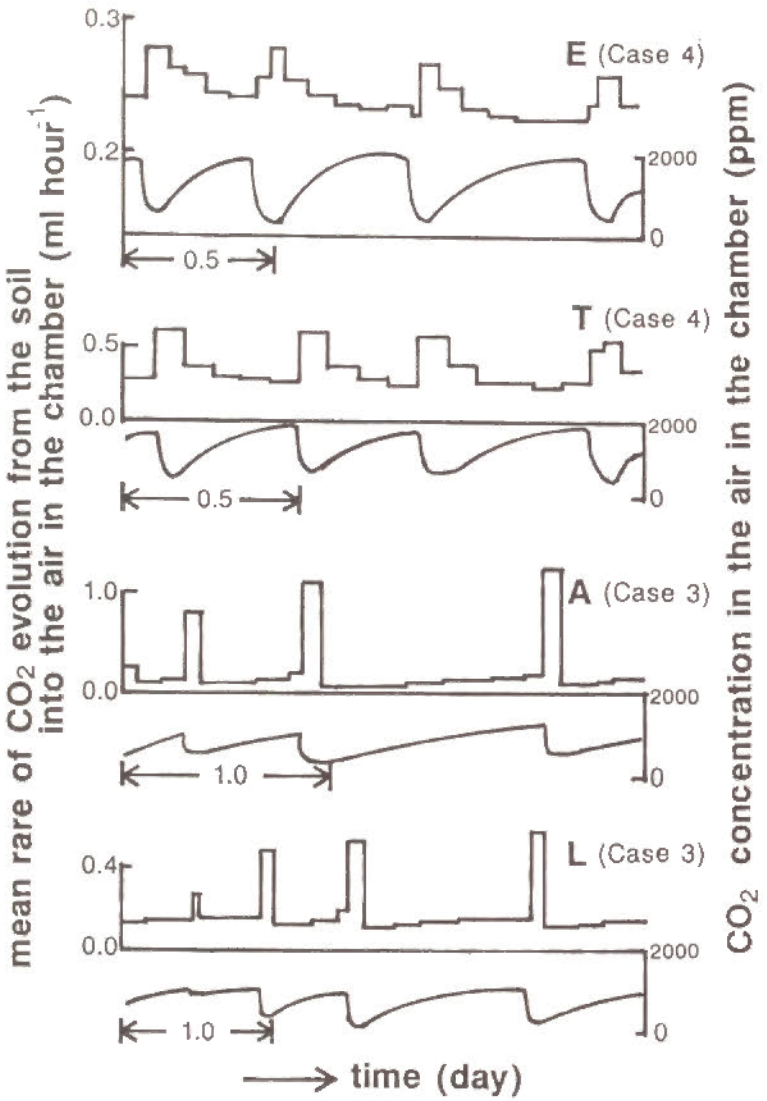

Fig.5-4. Changes of the mean rate of CO_2 evolution x and the concentration (partial pressure Pa). The each curve of concentration is below that of CO_2 evolution, respectively. T: 2/3kg of andosol packed tightly, L: 1/4kg of andosol packed loosely, E or A: light clay soil amended with glucose and packed in a beaker as shown in Figure 5-3, and covered with a polyethylene film (E) or supplied with activated alumina (A). CO_2 concentration was regulated by changing the volume of CO_2 free air flown into the chamber so as to vary the concentration widely, and the mean rate of CO_2 evolution was resultant parameter.

andosol packed in the chamber as tightly as in the field to suppress CO_2 diffusion. These are typical cases of concentration dependence (i.e. CO_2 evolution was suppressed by CO_2 concentration).

The soil used in the experiment shown in Figure 5-4L (Case 3) was the same andosol, but the soil was packed loosely. In this case, a high CO_2 concentration did not suppress CO_2 evolution. But, when CO_2 concentration in the chamber was decreased, CO_2 evolution was stimulated because CO_2 sorbed by soil under a high CO_2 concentration was liberated under a low CO_2 concentration. A similar result is shown in Figure 5-4A (Case 3). In this experiment, because activated alumina was used as a CO_2 adsorbent, stimulation of CO_2 evolution at a decreased CO_2 concentration was more than that in Figure 5-4L. Under laboratory conditions, a stimulation as observed in Figure 5-4L was more or less observed in most of the measurements.

As discussed above, concentration dependence is caused by CO_2-sorption by the soil and modified by CO_2 diffusion in the soil, though details of the phenomenon under various conditions are not known. A study of CO_2 sorption by the soil will be reported in a chapter to follow.

Summary

Concentration dependence of CO_2 evolution from

soil was studied under field and laboratory conditions.

Under field conditions, when CO_2 concentration is measured with an infrared gas analyzer (IRGA) in a small and column-shaped chamber placed on the ground, the relationship among CO_2 concentration $\underline{c}$ (m^3m^{-3}), time $\underline{t}$ (hours), height of the chamber $\underline{h}$, a constant rate of soil respiration $\underline{v}$ ($m^3m^{-2}hours^{-1}$) and an appropriate constant $\underline{k}$, may be expressed by the following equation.

$d\underline{c}/d\underline{t}=\underline{v}/\underline{h}-\underline{k}(\underline{c}-\underline{a})$ ($\underline{c}=\underline{a}$ at $\underline{t}=0$)

Most of the data of measured CO_2 evolution fitted this equation. But the applicability of this equation is limited to the data to which a linear equation is not appropriate because the estimated value of v has a larger error than that estimated by a linear regression analysis, as found by a computer simulation.

The concentration dependence as shown above and some other variations may be elucidated by a sorption/diffusion model, i.e. it may be caused by CO_2-sorption by the soil and modified by the condition of CO_2 diffusion in the soil.

CHAPTER 6

SEMI-REVERSIBLE CO_2-SORPTION IN THE SOIL

In Chapter 5, a CO_2 sorption/diffusion model in the soil was briefly described. In this chapter, CO_2 sorption in soil will be discussed.

CO_2 sorption in the soil is widely variable and is caused by many processes, e.g. absorption by alkaline substances, dissolution by soil water, stock in soil pores resulting in high partial pressure, and adsorption by surfaces of soil particle. Furthermore, the rate of these processes is also an important factor in determining the volume of CO_2 sorbed in the soil. The objective of this study is not to elucidate the mechanism of the sorption, but to determine the reversibility (using the time scale of 'hours') and concentration dependence of CO_2 sorption in the soil.

Materials and Methods

The CO_2 input/output of the soil was measured using the automatic setup described previously (Chapter 3). The soil, in which biological activity was strongly suppressed, was placed in a chamber of the setup. CO_2 concentration in the chamber was controlled by the introduction of air without CO_2 or that with a known concentration of CO_2.

Unless otherwise stated, the measurement was

made at 20°C in water baths in an air-conditioned room, the flow rate of the air was approximately 1 liter / hour, and the soils examined were air-dried and passed through a 65 mesh sieve.

To dry or moisten soil samples, the samples were put in evacuated and sealed chambers with anhydrous $CaCl_2$ or distilled water.

The influence of alkali on the CO_2 sorption was determined by blending the soil and 1N-KOH solution in the ratio of 1:5 in a reciprocating blender for 2 hours. The soil sample was then taken out from the mixture by decantation or centrifugation, dried at 80°C, pulverized and sieved once again with a 0.2 mm sieve.

To remove organic matter, the soil sample was boiled with H_2O_2 solution.

Results and Discussion

Figure 6-1 shows the sum of CO_2 evolution from an autoclaved (120°C 30 min.) soil, which was the same andosol described in Chapter 5 and which contained moisture almost corresponding to the field capacity (38%). Curves 1 and 3 show CO_2 evolution from the soil during a decreasing process of CO_2 concentration, and curves 2 and 4 show CO_2 sorption by the soil during a process of CO_2 increase. As shown in the figure, CO_2 sorption by the soil is reversible.

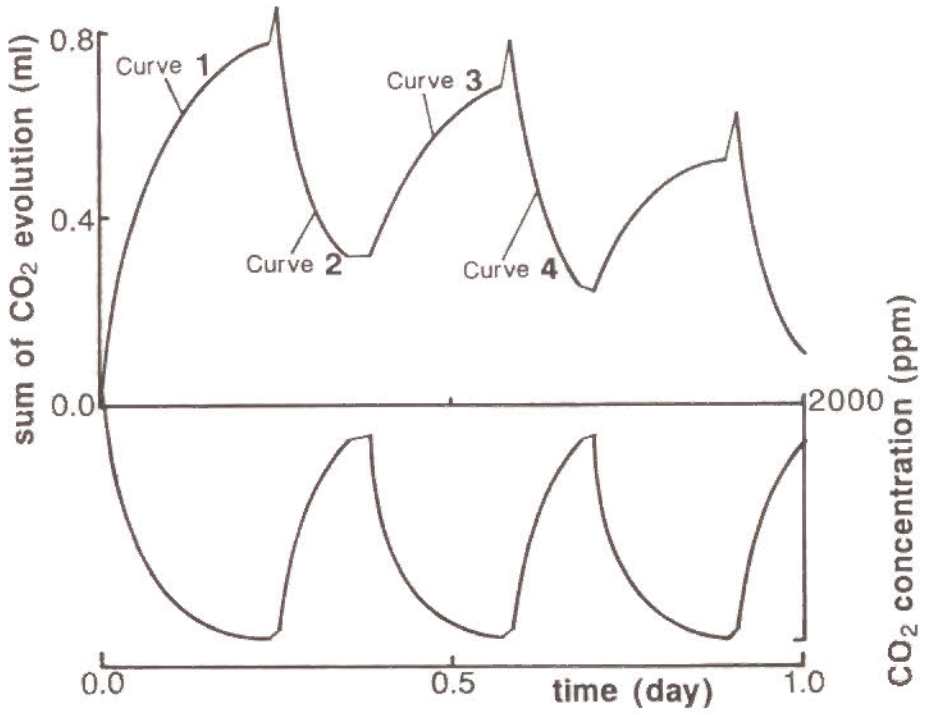

Fig.6-1 Changes of sum of CO_2 evolution from an autoclaved soil as regulated by CO_2 concentration in the chamber. CO_2 concentration in the chamber was controlled by introduction of air with a known CO_2 concentration. The measurement was made at 25°C

The relationship between CO_2 concentration and a volume of CO_2 sorption (the sum of negative CO_2 evolution) by the soil is shown in Fig.6-2, where curves 1, 2, 3 and 4 correspond to those in Fig.6-1.

The data used in Fig.6-2 are the same data as in Fig.6-1.

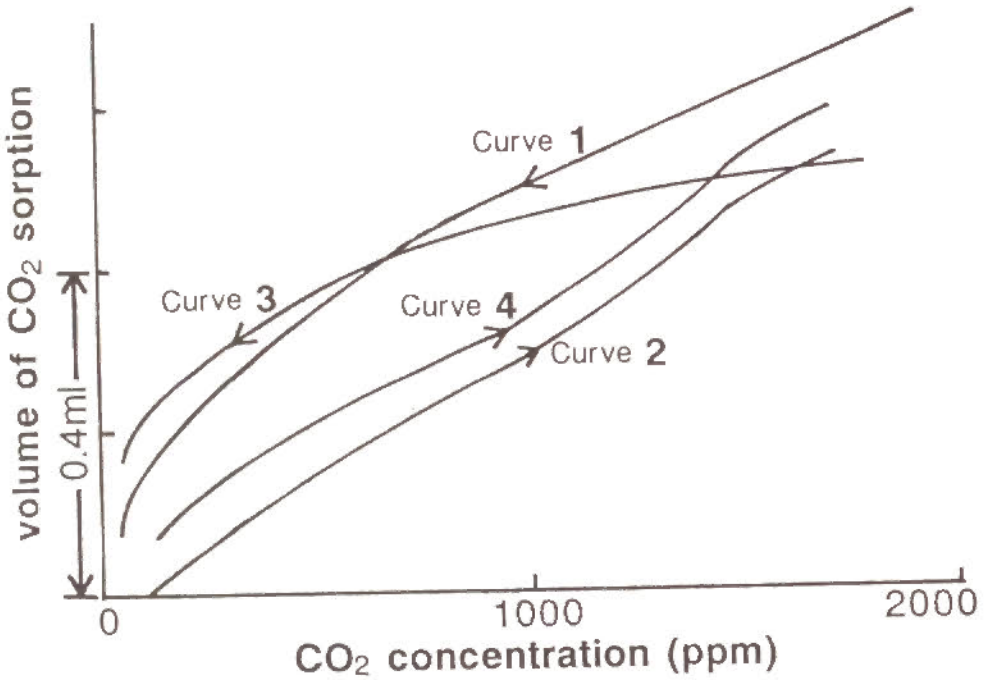

Fig.6-2 Relationship between volume of CO_2 sorption (sum of negative CO_2 evolution) and CO_2 concentration in the chamber. The same data as in Fig.6-1 were used.

Figure 6-3 shows similar curves for the air dried soil taken from the C horizon of an andosol. Reproducibility was good with air-dried and sieved (<0.2 mm) soil samples, but not with autoclaved wet soils. Similar curves were observed not only in the

soil samples but also in some powdery materials (e.g. activated alumina, activated carbon and aluminum hydroxide).

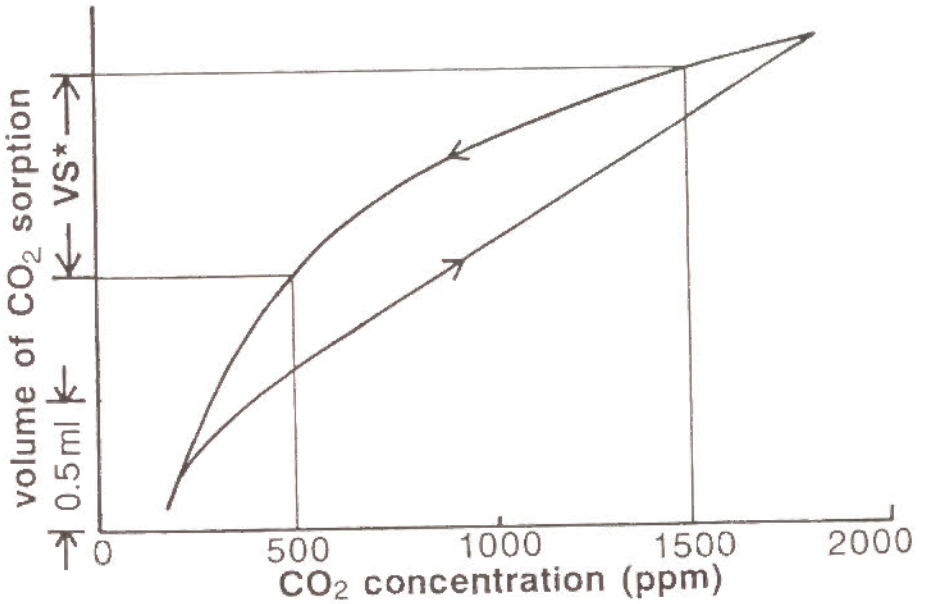

Fig.6-3 Relationship between volume of CO_2 sorption (sum of negative CO_2 evolution) and CO_2 concentration in the chamber and definition of a 'volume' of CO_2 sorption **VS**.

The difference between sorption process and desorption process was observed and this is shown in Figs.6-2 and 6-3. The difference may observed when

sorption at a certain concentration does not reach an equilibrium and/or the sorption may have a character of hysteresis. The details of the problem involved in this curve is not yet known. But, to determine the nature of CO_2 sorption by soils of various types and characters, we define a 'volume' of CO_2 sorption to facilitate a comparison between samples. The 'volume' is the difference between the volume of CO_2 sorption at the chamber CO_2 concentration of 1500 ppm and at 500 ppm, as shown in Fig.6-3. Reproducibility of the measured 'volume' under the condition described above was fairly good.

Table 6-1 Relationship between 'volume' of CO_2 sorption and soil moisture

soil name	dried with $CaCl_2$		air dried		moistened	
	soil V.S.	moist.	soil V.S.	moist.	soil V.S.	moist.
Aira	0.80	6.2	0.72	7.5	0.55	10.8
Shichihonzakura	1.22	9.6	1.23	11.7	1.04	14.6
Imaichi	1.92	18.9	1.71	23.6	1.45	32.0

V.S., 'volume' of CO_2 sorption (ml / 20g wet soil); soil moisture, % ; Moisture tension of the soil dried with $CaCl_2$, dried in air or moistened can be estimated about >300, 30 or 3> MPa, respectively. Aira, Imaichi and Shichihonzakura are subsoils of andosols.

Table 6-1 shows the relationship between the CO_2 sorption volume and the soil moisture. A little difference of the sorption volume was observed in one soil. But, among the different soils under the same moisture condition i.e. at the same water tension, the more the moisture, the higher the 'volume' of CO_2 sorption. Consequently, it is possible to conclude that the 'volume' of CO_2 sorption is affected by a water retentivity of the soil, not by a moisture content itself.

Table 6-2 Influence of alkaline and/or H_2O_2 treatment on 'volume' of CO_2 sorption

soil (horizon) or powder	control	0.1N-KOH	H_2O_2	H_2O_2 0.1N-KOH
mineral soil (AP)	0.0 (6.4)	0.9	0.0	2.4
mineral soil (C)	0.1 (4.7)	2.8		
kaolin	0.0 (4.0)	2.0		
andosol (A)	0.0 (5.2)	0.7	0.0	1.1
andosol (C)	0.7 (5.6)	3.1	0.5	3.3
$Al(OH)_3$	0.4 (9.4)	2.0		

* 'volume' of CO_2 sorption (ml / 20g air dried soil); in (), pH(H_2O). The mineral soil (AP) is a light clay soil described in Chapter 4 and 5, and has an organic carbon content of 1.3%. The andosol (C) is Aira horizon described in text.

When solutions or powders of alkali or alkaline carbonates were used, reversible CO_2 sorption was not observed. With soils, filter paper or kaolin, CO_2 sorption was not observed, but when these samples were mixed with a small amount of $KHCO_3$ or treated with a KOH solution, they exhibited a reversible CO_2 sorption. For example, Table 6-2 shows a result of the 0.1N-KOH treatment, where the 'volume' of CO_2 sorption of the soil or the mineral was increased by the treatment.

Table 6-2 also shows the influence of the removal of organic matter by boiling in H_2O_2 solution. The 'volume' increase in the surface soils was less than that in subsoil with 0.1N-KOH. But the H_2O_2 treatment for the removal of organic matter increased the 'volume' after KOH treatment. Concentration dependence of CO_2 evolution in the field, which was often observed on the exposed subsoil, may be related to the result of this experiment, i.e. the absence of organic matter.

The total quantity of Lewis base located on the soil-air interface may be related to the capacity of reversible CO_2 sorption of an air-dried soil at low atmospheric partial pressure of CO_2. The quantity of the Lewis base in aqueous solution can be fairly well correlated with variable charge of the soil, but the nature and the quantity of the functional groups concerned under air-dried conditions are currently unknown.

Summary

Semi-reversible CO_2 sorption on the air-dried and 0.2mm sieved soils was studied with the same setup as shown in Chapter 3.

A large amount of CO_2 sorption on andosol subsoil was observed. From the soil samples, it was possible to conclude that the 'volume' of CO_2 sorption is affected by a water retentivity of the soil, not by a moisture content itself. An increase of sorption with pH rise was observed especially on subsoils or on soils where organic matter has been removed by H_2O_2 treatment.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Measurement of CO_2 evolution with infrared gas analyzer (IRGA) is easy, rapid and accurate, because CO_2 concentration in the object chamber is directly and rapidly measured. But the rate of CO_2 evolution from the soil is still very variable, and it is important to interpret the data of CO_2 evolution rightly.

As shown in Chapter 2, even fundamental factors, such as soil moisture and presence of organic matter, only minimally affect CO_2 evolution from the soil in the field. However, the effect of other factors (unknown or error factors) on CO_2 evolution are greater. This exemplifies the difficulty of interpreting the data on the influence of factors controlled by researchers, e.g. the application of agrochemicals.

The reason for the minimal effect of the factors cited above on soil respiration could be either species-specificity or location-specificity of the treatments. A certain agrochemical may affect only a certain group of organisms while an incorporation of organic matter may enhance the activity of organisms that have ready access to the organic matter.

On the contrary, soil temperature and application of chloropicrin fumigant strongly affected CO_2 evolution, because these factors could affect almost the whole biota.

The activity of soil organisms could be directly affected by soil inorganic materials acting as a barrier between an organism and a substance which either inhibits or nourishes it. The rate of CO_2 evolution from soil was not only affected by the activity of soil organisms but also by the physical and chemical characters of soil inorganic materials. The latter may also be a source of error in the measurement of soil biological activity. Accordingly, CO_2 sorption and diffusion in the soil are important problems for the measurement of the rate of CO_2 evolution from the soil.

Quantitative, simulated experiments on the problems of CO_2 sorption/diffusion and a special experiment on CO_2 sorption by air-dried soils were conducted (Chapters 5 and 6). The influence of these problems on various types of measurement of CO_2 evolution has not yet been fully evaluated. The author holds the view that CO_2 sorption is not so serious in experiments with ordinary soils in Japan.

The method of measurement of CO_2 evolution from the soil and the factors affecting it were studied in the present work. However, there still remain important, interesting and unsolved questions.

The alkaline absorbent method is still an impor-

tant method for the measurement of CO_2 evolution from the soil especially in vast experimental sites such as forest, rangeland, and so on. However, a reexamination of the method is desirable for more accurate and convenient evaluation of the rate of CO_2 evolution under field conditions, in comparison with IRGA methods.

The unknown factors which affect the variation of the CO_2 evolution data are also important subjects for further investigations. The spatial variability of the activity of soil organisms is especially relevant to soil sampling methods, and it is also relevant to mathematical and ecological investigations of soil ecosystems.

An investigation of CO_2 sorption and diffusion in the soil is also required. This is not only for a more accurate measurement of CO_2 evolution, but also for the study of soil aeration. Because sorption is concerned with the interaction between soil minerals and C=O bonds, an investigation about this may involve soil mineralogy and humus studies.

CO_2 evolution from the soil is a pathway of CO_2 flux into the above-ground part of ecosystem. Plants could take up CO_2 from the soil along with water. This may be another main pathway of CO_2 flux and an error factor of the measurement of soil microbial activity. In our experiment, changes of CO_2 evolution in the field within a 24-hour period were not affected so strongly by the soil temperature. One of the

reasons of this may be the influence of plant activity on CO_2 evolution. An easy method for a measurement of this flux in the field should be developed.

Conclusions

1. The rate of CO_2 evolution from the soil is highly variable and the variation is caused not by the method of measurement but by the variation of the activity of soil organisms themselves.
2. The factors affecting almost the whole biota, such as the soil temperature, strongly affects the rate of soil respiration.
3. The factors having either species-specificity or location-specificity, such as an application of agrochemicals or incorporation of organic matter, little affects the rate of soil respiration.
4. CO_2 sorption and diffusion in the soil are important factors for a more accurate measurement of the rate of soil respiration.

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